DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA

Gut Microbiota

A Review of Fecal Microbiota Transplantation, *Clostridium difficile* infection, Diet and Obesity

António Eduardo Cabrita Figueiredo

Orientação Científica: Dr. Robert Badura

Clínica Universitária de Doenças Infecciosas e Parasitárias

Prof.ª Doutora Emília Valadas

Faculdade de Medicina da Universidade de Lisboa

2015-2016

Index

List of Abbreviations

CD - *Clostridium difficile*

CDI - *Clostridium difficile* Infection

CONV - Conventionally raised

DM2 - Diabetes Mellitus type 2

FGF21 - Fibroblast Growth Factor 21

Fiaf - Fasting-induced adipose factor

FMT - Fecal Microbiota Transplantation

GF - Germ-free

GPCR TGR5 - G-Protein Coupled Receptor TGR5

HF - High-fat

HGC - High-gene count

HS - High-sugar

IBD - Inflammatory Bowel Disease

IL - Interleukin

LGC - Low-gene count

LPS - Lipopolysaccharide

MAC - Microbiota-accessible Carbohydrate

NOD - Non-obese, diabetic

qPCR - quantitative Polymerase Chain Reaction

rCDI - recurrent *Clostridium difficile* Infection

RELM - Resistin-like molecule

RNA - Ribonucleic Acid

rRNA - ribossomal Ribonucleic Acid

SCFA - Short Chain Fatty Acid

SFB - Segmented Filamentous Bacteria

T1D - Type 1 Diabetes Mellitus

TG - Triglyceride

TLR - Toll-like Receptor

Treg - Regulatory T cells

USA - United States of America

Abstract

The study of microbiota, the collective term for the microbial communities that inhabit us, is an increasing area of interest for the scientific community. *Clostridium difficile* infection is a prototypic case of deregulated interaction between the host and its gut microbiota, triggered by an environmental stimulus: antibiotics. Studies concerning the treatment of recurrent *Clostridium difficile* infection are demonstrating the ability to introduce different microbiota that resembles the donor's stool through Fecal Microbiota Transplantation.

Review of recent literature on gut microbiota revealed:

1) The complex interactions between different bacterial species, the environment and the host play a larger than expected role in regulating the host's metabolism and immune system. Understanding this relationship will most likely be useful in deciphering the pathophysiology of diseases with an emergent microbiota-related component.

2) Healthy microbiota is probably linked to hosting a diverse set of bacteria rather than specific bacterial species.

3) Diet is a predominant environmental factor, driving alterations in gut microbiota composition and function. Diet-induced extinctions might be unable to be recovered without specific intervention.

4) Although causality has not been clearly established, altered microbiota might play a role in the establishment of obesity. Study of this intricate relationship may reveal new medical approaches.

Resumo

O estudo das comunidades de micro-organismos que habitam o ser humano constitui uma área de interesse crescente comunidade científica. A infecção por *Clostridium difficile* exemplifica uma interacção desregulada entre o hospedeiro e a sua flora intestinal, desencadeada por um estímulo ambiental: antibióticos. Publicações recentes, incidindo sobre o tratamento da infecção por *Clostridium difficile* recorrente através da transplantação de micro-organismos fecais demonstram a capacidade de introduzir na comunidade do beneficiário, micro-organismos semelhantes aos das fezes do dador.

A revisão da literatura recente sobre a flora intestinal revelou que:

1) As interacções entre as diferentes espécies de bactérias, o ambiente e o hospedeiro desempenham um papel importante na regulação do metabolismo e do sistema imune do hospedeiro.

2) A composição de uma comunidade microbiana associada à saúde está associada à diversidade de espécies e não a espécies bacterianas específicas.

3) A dieta é um fator ambiental predominante, levando a alterações na composição e função dos micro-organismos intestinais. Extinções de espécies bacterianas induzidas pela dieta poderão ser incapazes de ser recuperadas sem uma intervenção dirigida.

4) A causalidade entre alterações da flora e o desenvolvimento de obesidade não está claramente estabelecida. No entanto, novas abordagens médicas poderão surgir através do estudo desta relação.

0) Intestinal Flora Dysfunction in *C. difficile* infection: A successful microbiota-directed therapy

What is *Clostridium difficile* Infection?

Clostridium difficile is a Gram positive, anaerobic bacillus that is capable of forming spores and producing toxin. (1) In a susceptible host, ingestion of its spores allows the colonization of the large gut causing a gastrointestinal infection termed *Clostridium difficile* Infection (CDI).

Initially termed clindamycin-associated pseudomembranous colitis (2), CDI has been associated with almost all classes of antibiotics. Third generation cephalosporins and fluoroquinolones are linked to the highest incidence of CDI(3) and even metronidazole can incite the disease. (4)

Colonization is prevented by barrier properties of the fecal microbiota; weakening of this resistance by antibiotics is the major risk factor for this disease. Other documented risk factors for infection include advanced age, inflammatory bowel disease, organ transplantation, chemotherapy, antiacids or proton pump inhibitor use, chronic kidney disease, immunodeficiency, and exposure to an infant carrier or infected adult. *C. difficile* infection is associated with severe illness, infection-related mortality of 5%, and all-cause mortality of 15 to 20%. (5,6)

Although antibiotics are the most frequent trigger for this infection, treatment of most CDI cases is based on oral antibiotic therapy. In non-recurrent infection, oral vancomycin or metronidazole are usually used for moderate to severe cases, with newer treatments such as fidaxomicin playing a more limited role due to its monetary cost. (5,7)

Antibiotic treatment for an initial *Clostridium difficile* infection typically does not induce a durable response in 15 to 26% of patients. (8) A new episode of CDI within 8 weeks after the resolution of symptoms from a previous episode, following appropriate treatment, defines recurrent CDI (rCDI). Both relapses and reinfections are covered in this broader category. (7)

The estimated efficacy of antibiotic therapy for a first recurrence is 60%, a proportion that further declines in patients with multiple recurrences. (8) It is estimated that 10% (9) to 50% (10) of recurrent CDI cases may be attributable to reinfections rather than recurrence of the initial infection.

What is Fecal Microbiota Transplantation?

Historic perspective

Currently, the only existing non-antibiotic treatment for recurrent CDI that is recommended internationally is fecal microbiota transplantation. The accepted protocol is described in the annex section. The earliest reference of the use of human feces as a medical treatment dates back to fourth century in China where it was used to treat patients with food poisoning or severe diarrhea. (11) The first use of fecal enemas in humans for the treatment of pseudomembranous colitis was reported in 1958 in a four-patient case series. (12) *Clostridium difficile* as the causative agent of most pseudomembranous colitis was not identified until 1976. (13) A case report in which fecal enemas were used to successfully treat a patient with rCDI was published in 1984. (14,15) In the early 2000s the appearance of the BI/NAP1/027 strain of *C. difficile* was associated with widespread epidemics of CDI. This strain is characterized by high-level fluoroquinolone resistance, efficient sporulation, markedly high toxin production, and a mortality rate three times as high as that associated with less virulent strains. (5) Following this series of epidemics, the number of rCDI cases increased which led to the use of FMT in cases in which no other therapy was effective, prompting an increase of studies on FMT.

Results

The first multicenter long-term follow-up for FMT in 2012 reported a resolution rate of rCDI of 91% for primary cure rate (resolution of symptoms without recurrence within 90 days of FMT) to 98% for secondary cure rate (resolution of symptoms after one further course of vancomycin with or without repeat FMT). No adverse effects directly related to the transplantation were reported. (15) The results of a randomized and controlled trial from 2013 for fecal microbial transplantation demonstrated that administration of vancomycin followed by an infusion of donor feces delivered by nasoduodenal tube was superior to vancomycin alone for recurrent CDI. The patients in the infusion group had an 81% resolution rate after first infusion contrasting with the 31% rate in those receiving vancomycin alone. (8)

The overall recurrence rate (defined as laboratory-confirmed reappearance of C. difficile and diarrhea after resolution of the previous episode) after completion of FMT therapy is 5.5%. Early recurrence rate of older patients is higher than that of younger patients, which in turn leads to reduced frequency of primary cure rates in this group. Most recurrences are associated with antibiotic use following infection apparently

unrelated to the procedure. (16) The European Society of Clinical Microbiology and Infectious Diseases now strongly recommend the usage of fecal transplantation in combination with oral antibiotic treatment for multiple recurrent CDI unresponsive to repeated antibiotic treatment. (7)

How FMT acts on *C. difficile* infection: restoration of host-microbiota interaction

C. difficile pathogenesis begins with ingestion of its spores. They are able to resist stomach acids and digestive enzymes, reaching the cecum and colon unaltered. In a suitable host *C. difficile* spores then germinate into vegetative cells, leading to toxin production and finally disease. (17)

Antibiotics alter bile acid metabolism

Antibiotic exposure triggers a change in the composition of the gut microbiota which in turn causes a shift in the gut environment: from a medium unfavorable for *C. difficile* colonization to a medium that includes its required germinants and substrate. Out of all variables, bile metabolism seems to play a critical role in the development of infection.

Analysis of the metabolic fecal products of mice treated with a 10-day course of broad spectrum antibiotic showed an increase in bile salts including taurocholic acid and other tauro-conjugated bile acids. Also, the concentration of secondary bile acids deoxycholate and cholate was reduced to undetectable levels. Other significant changes included increases in amino acids required for *C. difficile* germination as well as increases in sugar alcohols (manitol and sorbitol) and decreases in the levels of SCFAs. (18) Similarly, metabolic analysis of CDI patients showed high concentrations of primary bile acids and bile salts, while secondary bile acids were nearly undetectable. In contrast, post-FMT fecal samples contained mostly secondary bile acids, as did non-CDI donor samples. (19)

Changes in bile metabolism trigger *C. difficile* Infection

Bile salts are well known germinants of *C. difficile* spores. The germination-specific receptor for taurocholic acid (a bile salt), CspC, was only recently discovered**.** (20) They serve as a signal to the spore that it has reached an anaerobic environment, the intestinal tract. (17) Taurocholic acid is rarely available to allow *C. difficile* to germinate in a healthy gut given the constant catabolism of bile salts by the normal flora. (21) On the other hand, secondary bile acids inhibit *C. difficile* outgrowth or germination, a function that has been demonstrated in both *in vitro* (22,23) and *in vivo* (24) studies.

The colonic flora's role in bile metabolism

There are a number of mechanisms by which colonic flora metabolize bile salts, rendering them unable to stimulate *C. difficile* germination: deconjugation by bile salt hydrolases and reduction by reductive hydroxylation reactions. Bile salt hydrolases catalyze the hydrolysis of amide bond in the conjugated bile salts, then form the deconjugated bile acids (mainly cholic and quenodeoxycholic) until free amino acids are dissociated. These primary bile acids may afterward undergo dehydroxylation and get converted into secondary bile acids (deoxycholic and lithocholic) after a series of changes. Bile hydrolysis is quite common for different gut bacteria and is hypothesized to be beneficial to commensal bacteria by liberating nutrients and conferring tolerance to the antimicrobial properties of bile acids. On the other hand, a more restricted group of bacteria have the ability to convert primary bile salts into secondary bile acids through 7-alpha-dehydroxylation. (21,25)

Transplant of specific species confer resistance to *C. difficile* Infection

It has been postulated that by increasing the relative abundance of members of these bacterial families, it is possible that FMT increases 7-dehydroxylation activity, leading to increased secondary bile acids and decreased primary bile acids. (19)

Current evidence suggests that resistance to *C. difficile* infection might be associated to loss of specific bacterial features following antibiotic treatment rather than overall loss of gut microbiota diversity. By comparing antibiotic–treated or untreated mice in two independent studies, investigators found that overall community size doesn't significantly differ between the two groups. (17,18) Also, high bacterial diversity was not necessarily associated with protection against *C. difficile* and low diversity was not necessarily associated with susceptibility to the growth of *C. difficile*. (17)

To determine which group of bacteria conferred protection to infection, investigators then correlated loss of specific bacterial taxa with development of infection in 24 allogeneic hematopoietic stem cell transplantation patients diagnosed with CDI out of which 12 were carriers without clinical infection.

The resulting group of bacteria was then confronted with a similar group obtained from mice, resulting in a final group of species that correlated in both mice and human with resistance to infection. This group of bacteria was comprised primarily by Clostridium cluster XIVa, including the species with the strongest resistance correlation, *Clostridium scindens*.

To evaluate causality they then performed adoptive transfer of the resulting consortium or *C. scindens* alone before inducing *C. difficile* infection. The results were a significant decrease in *C. difficile* colony-forming units as well as associated weight loss and mortality in both arms when compared to control. This bacterial transfer was precise and engraftment did not alter other aspects of microbiota structure compared to control, including density and biodiversity.

The mechanism by which *C. scindens*, a bile acid 7-dehydroxylating bacteria, appears to confer resistance to infection is through conversion of stimulatory combinations of bile acids into inhibitory combinations of bile acids. This study showed that a fraction of the intestinal microbiota as precise as a single bacterial species can confer infection resistance to *C. difficile*. (17,26)

There is a risk in manipulating bile acids directly. Increased levels of some secondary bile acids have been linked to gastrointestinal cancers (27) or cholesterol gallstone disease. (25)

Adverse Effects of Fecal Microbiota Transplantation

The most frequent adverse events reported after FMT were mild to moderate bloating, abdominal pain and diarrhea. These were short-term and subsided after a few days. (16) Altogether, the risks associated with FMT are few. Although the transmission of an undetected or unidentifiable pathogen from the donor is a possibility, there are no known reports of serious infectious complications resulting from fecal microbial transplantation that was performed with appropriate donor screening. (5) There have been concerns in using fecal microbiota transplantation from obese donors following a case-report that shows unintentional weight gain after the transplantation. (28) It is important to note that some recipients of FMT developed new immune conditions in some studies, such as rheumatoid arthritis, peripheral neuropathy, Sjögren's syndrome, and idiopathic thrombocytopenic purpura. It is not known if they were related to the FMT. (11) Disease flares in patients with a history of IBD treated for CDI have been reported in the literature. A case report published in 2013 presented a case of a patient treated with FMT for recurrent CDI which resulted in a flare of a long time quiescent ulcerative colitis soon after the treatment. Although there is a noteworthy amount of patients treated for IBD with a FMT, it is important to expect unforeseeable consequences in this population. There are risks associated with the procedure to administer the FMT by colonoscopy, sigmoidoscopy, or the upper route when aspiration could occur. Deaths have occurred as a direct result of FMT. A case of toxic megacolon

and septic shock following the procedure was reported. Two report cases of fatal aspiration pneumonia have also been reported, both of which happened in patients sedated for the procedure, upper endoscopy and colonoscopy.(29,30)

Changes in microbiota following FMT

The dysbiotic state found in rCDI patients is characterized by a large expansion of Proteobacteria (primarily members of the order Enterobacteriales, which contains the family *Enterobacteriaceae*), and FMT is associated with reemergence of dominance by members of the Bacteroidetes and Firmicutes phyla.

Immediately after FMT, there is a rapid normalization of bacterial fecal sample composition from a markedly dysbiotic state to one representative of normal fecal microbiota. (31) Healthy bacterial interaction networks which were absent beforehand can be detected immediately after the transplant, testifying the community-level changes that occur. The changes in microbiota adopt donor-specific signatures that are maintained over time. (32)

The first characterization of long-term microbiota changes in patients after FMT was performed by Song *et al*. Samples from fourteen pairs of healthy donors and rCDI patients treated successfully by FMT were collected up to one year after the transplantation. These authors argued that, although there was a disappearance of diarrheal symptoms immediately after FMT, as changes in Firmicutes continued to occur, full microbiota recovery from rCDI might take much longer than expected.(33)

On the other hand, a more recent study highlighted that the drifts in microbiota composition observed following FMT fall under the normal microbial variation of healthy adults. By comparing donor and recipient sample composition and variability they found that these are characterized by highly dynamic shifts that nonetheless remain within the compositional range of normal fecal microbiota. This observation is consistent with known rapid responsiveness of the fecal microbiome to environmental inputs, such as dietary variations, and drifts in microbiota composition over time in healthy individuals. The dynamic nature of intestinal microbiota is an intrinsic property, which should be taken into account when considering how therapeutic interventions, including FMT, impact its composition over time. (31)

Further considerations:

After establishing the efficacy of FMT, the emphasis of current research is now directed towards the standardization of the technique, assuring its safety and finding new indications.

Patient-donor relationship: There isn't a significant difference in cure rate or recurrence rate between patients treated with patient-related donors or unrelated healthy donors. (16)

Fresh vs. Frozen stools: The viability of fresh stools is limited, being usually estimated at up to 6 hours. (34) A double-blind randomized clinical trial from 2016 to determine whether frozen FMT administered through enema is non-inferior to fresh FMT for patients with recurrent or refractory CDI concluded that frozen FMT did not result in a worse proportion of clinical resolution of diarrhea. (35) The use of frozen FMT ameliorates the risk of transmitting pathogens from the donor to the recipient as it allows for the proper screening of the sample before the procedure. It also permits the use of FMT in non-specialized centers without the required technical facilities for sample screening.

Route of Administration: Although no random controlled study has established any route of administration as preferred, a meta-analysis from 2015 found that the primary cure rate of the lower gastrointestinal route group was significantly higher than that of upper route (81% vs. 93%). (16) Even so, a small randomized, controlled pilot study from 2014 using frozen inoculum concluded that nasogastric tube administration appeared to be as effective as colonoscopic administration. (36) In another study, using oral administrated capsules containing frozen FMT from unrelated donors achieved a resolution rate of diarrhea of 70% after primary treatment and greater than 90% with subsequent treatments. (34) This result matched a smaller study's result of cumulative clinical cure rate of 89% for orally administered capsules. (37) Using capsules as a form of administration might prove useful in critical patients unable to undergo more invasive techniques.

Cost-effectiveness: A cost-effective analysis on colonoscopic administration of FMT compared with vancomycin evaluating the use of FMT at the third recurrence of CD diarrhea found the first to be the most cost-effective treatment strategy. (38)

Non-recurrent CDI: It has been suggested that FMT might have a prospective role in the treatment of non-recurrent CDI. In a series of cases, 14 subjects with severe non-recurrent CDI refractory to medical treatment of vancomycin and metronidazol obtained a cure rate of 79% after intestinal microbiota transplant. More studies are required to clarify this indication. (39)

Bacteriotherapy: The unappealing FMT might be soon replaced by bacteriotherapy, a more specific, controllable, and possibly effective treatment. Bacteriotherapy is not a

new concept. In 1989 a series of 5 patients was treated for rCDI by rectal infusion of a bacterial mixture which resulted in the prompt loss of *C. difficile* and its toxin from the stools of all patients following treatment. (40)

An upcoming study "RePOOPulating the Gut" has established proof of concept for this principle. The authors developed a synthetic stool mixture by culturing a stool sample obtained from a healthy donor and collecting bacterial isolates. The stool substitute consisted in a preparation of 33 different intestinal bacteria. It was administered to two patients with rCDI and a clinical cure was achieved. The patients remained symptom free at 6 months of follow-up even after several courses of treatment with antibiotics for unrelated infections. Advantages of a synthetic stool mixture are that the composition of the administered bacteria is known and can be controlled, the procedure is reproducible and other pathogens such as viruses, fungi and *archaea* can be excluded from the mixture. (1)

1) The Importance of Gut Microbiota

All of the surfaces of the human body that are exposed to the environment are colonized by microbes. Although only 20-60% of the microbes identified in different body sites have been cultured, recent technological advances such as 16S ribosomal RNA gene sequencing or whole-genome shotgun have allowed the characterization of microbial entire genomes and metabolic pathways directly from their natural source.

These advances have spiked the interest of the scientific community in studying the human microbiome. The microbiome is defined by a fluctuating collection of genes and gene products which is susceptible to alteration from environmental perturbations, such as antibiotic treatment and infection. Yeasts, *archaea* and viruses also form part of the human microbiome but most published studies have focused on host-associated bacterial communities. (41–43)

Initiatives such as the Human Microbiome Project (44) or the Metagenomics of the Human Intestinal Tract (45) try to establish a database for the normal human microbiome by analyzing hundreds of healthy donors.

Among body sites, the highest numbers of taxonomic units and genetic contents have been observed in stool samples. (45) The focus on the study of the human gut microbiome is supported by the fact that gut microbiota compose around 70% of the total microbiota found of the human body.

Composition of Gut Microbiota

The majority of human-associated bacteria fall within four phyla: the very prevalent Firmicutes, and Bacteroidetes and the least prevalent Actinobacteria and Proteobacteria. Each phylum contains many different bacterial species. (41) The Firmicutes phylum is composed of gram-positive, anaerobic, spore-forming bacteria that ferment simple sugars to produce a variety of SCFAs. Within Firmicutes, members from the *Lachnospiraceae* and *Ruminococcaceae* family make up 50 to 70 percent of the colonic bacterial population. Bacteroidetes are gram-negative, anaerobic, nonspore-forming bacteria that are enriched with enzymes to degrade carbohydrates. (46)

Curiously, even the more abundant species in healthy individuals such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Bacteroides uniformis* can present at <0.5% relative abundance in some healthy individuals. (47)

We share a core functional microbiome but not a core group of microbiota. Core functions of the gut include carbohydrate and amino acid metabolism, and these can be performed by several species of microorganisms. Some functions aren’t a part of the

core: pathogenicity islands, vitamin and drug catabolism, motility and nutrient transporters are pathways that vary between individuals. (47)

Functions of the Gut Microbiota

There is a complex symbiotic relationship between gut microbiota and its host. The microbiota carries out specific functions unable to be performed by the host.

Production of Short Chain Fatty Acids

Bacteria play an important functional role by metabolizing the undigested carbohydrates, lipids and protein that reach the colon each day. They ferment complex indigestible carbohydrates and amino acids into short chain fatty acids (SCFAs), (acetate, butyrate, propionate and valerate). Members of the Firmicutes and Bacteroidetes account for most of the SCFA production with Firmicutes being the dominant producers of butyrate. (48)

While butyrate is used as an energy substrate for the colonic epithelium and can strengthen the colonic defense barrier by inducing secretion of protective factors and acidifying the colon lumen (49) (e.g. mucin, trefoil factors or antimicrobial peptides) (50,51) acetate and propionate are carried into the bloodstream and become available as energy substrate for peripheral tissues. (52) It is estimated that SCFA represent 5-10% of the host's daily energy intake. (53)

SCFAs play a vast number of roles regulating host homeostasis. They inhibit gut motility through stimulation of peptide YY production, allowing gut microbes to digest more polysaccharides. (54) They are involved in appetite control by interacting with hormone secreting gut endocrine cells (49) and may also play a role in leptin production by adipocytes. (55) Also, SCFAs play an important role in the suppression of the inflammatory response: butyrate, and proprionate actively promote the generation of Foxp3+ regulatory T (Treg). (51)

Butyrate in special takes part in immune modulation, cell cycle inhibition, induction of programmed cell death and cellular differentiation in a variety of cell types. Recently it has also been shown that butyrate is able to alter dendritic cell response to bacterial antigens, up-regulating IL-23 production. (51)

Lipid Metabolism

The intestinal flora also performs a fundamental role in lipid metabolism, specifically the metabolism of biliary acids, as described previously. Other than acting on the metabolism of fat and cholesterol, biliary acids are also able to modulated lipoprotein,

glucose, drug and energy metabolism by binding to the bile-acid-synthesis controlling nuclear receptor farnesoid X receptor and the GPCR TGR5. (52)

Other metabolites

Besides SCFAs gut microbiota also influences other important metabolites. Through modulation of the metabolism of tryptophan, a precursor of serotonin production, gut microbiota is able to influence behaviors and pathology that rely on serotonergic neurotransmission. (56) Recent research is now focusing on the gut-brain axis (57) to expand on host-microbiota interaction. Various B-vitamins and folate are biosynthesized by gut microbiota.(58)

Pharmabiotics

The gut microbiota is also relevant regarding drug metabolism. More than 30 drugs that made it onto the market have been identified as substrates for colonic bacteria**.** (59) The importance of microbiota metabolism of a pharmaceutical product cannot be overstated. In 1993 in Japan sorivudine, drug acting on herpes zoster infection, was introduced into the market. It was later discovered to be transformed by the gut microbiota into an inhibitor of 5-fluorouracil leading to toxic levels of this drug. Within forty days this interaction was responsible for the death of eighteen patients with cancer and herpes zoster leading to the withdrawal of sorivudine from the market. (60)

Microbiota and Immunity

Much of our current knowledge on the interactions between the microbiome and the immune system comes from studies on germ-free animals. These can be used in their sterile condition or they can be colonized by a single known species or consortium, originating gnotobiotic animals (from the greek: known life). (61)

The immune system modulates microbiota

Every day the intestine is exposed to an enormous microbial load. An important function of the immune system of the gut is to control and limit this exposure.

By stratifying the microorganisms into layers, the mucous produced by the gut limits the contact with the intestinal lining. In the colon, mucous exists in two different layers, an external microbial-rich layer and an internal less colonized layer. This separation is not only dependent of structural differences between layers but also dependent of secretion of specific IgA produced by lamina-propria B-cells as well as antimicrobial peptides, produced by the epithelial cells, namely RegIIIγ (61)

The uptake of antigen and microbes from the lumen of the gut and its delivery to underlying lymphoid tissue is performed by specialized epithelial cells, the M cells,

present in Peyer's patches. Bacteria that penetrate the intestinal barrier are engulfed by dendritic cells residing in the lamina propria and are carried alive to the mesenteric lymph nodes. However, these bacteria do not penetrate to systemic secondary lymphoid tissues. In this manner, there is an anatomical mechanism that limits the interaction of the microbiome with the systemic immune system. Limiting the innate response enables an increased systemic response by the host organism: Mice unable to produce IgA have an IgG response against the commensal flora. Mice with a limited TLR-response also produce IgG against commensal microorganisms.(61)

Defects in the immune system impact the microbiota in a manner that predisposes to disease. In several studies, microbiota obtained from genetically immune-compromised mice was notably altered which conditioned the development of pathology. After transferring it to wild-type mice, the associated diseased phenotype was transmissible. (61)

Host secretions not only limit the microbial load but can also change its composition, either by providing an ecological niche for specific bacteria or by selecting against specific bacteria. Mucin, a glycosylated protein covering the intestinal epithelium, is a specific growth substrate for many commensal gut microbes. (62)

Other host mechanisms relevant for microbiota modulation include intestinal motility and other secretions, such as Paneth cell secreted defensins, gastric acid or, the previously discussed biliary acids.

Microbiota modulates the host immune response

In the same way that the immune system modulates the gut microbiota, gut microbiota is known to modulate the development and homeostasis of the immune system**.** The development of various immune-related cells is influenced by gut microbiota. The gut mucosal immune system is the largest lymphoid site in body, and gut bacteria interact with lymphoid follicles of the gut mucosa, and regulatory and effector T cells. Dysbiosis, that is, disease-associated microbiota, changes the immune regulatory systems that normally manage inflammation in the gut, and is associated with immune-mediated disorders. (63)

Germ-free mice have no commensal intestinal microbiota and as such exhibit an underdeveloped immune system: reduced secretory IgA, defects in development of gut-associated lymphoid tissues, smaller Peyer's patches and mesenteric nodes. Also, an unorganized myenteric plexus and reduced intestinal motility. These changes translate in an increased susceptibility to infection in these animals. However, reintroduction of

intestinal flora in GF animals can restore the proper organization of the intestinal immune system. (57)

Beyond its role on the development of the immune system, the gut microbiota is able to actively change the immune response of its host. Much of what is known about microbiome–host immune interactions has been achieved from the study of single bacterial members: (64)

- Colonization of gnotobiotic mice with a consortium of mouse Clostridial strains, results in the expansion of *lamina propria* and systemic Tregs. (65)
- *Bacteroides fragilis,* a commensal bacterium produces polysaccharide-A which induces an IL-10 response in intestinal T cells which prevents the expansion of Th17 cells, actively inducing immunitary tolerance.(66)
- Colonization of mice with segmented filamentous bacteria (SFB) results in accumulation of Th17 cells in the lamina propria. This allows the adherence of SFB to the colonic mucosa and, by enhancing the immune response confers resistance to pathogen colonization.(67)

In all three of these examples, bacteria are able to enact a systemic influence on the host's immunity.

There is also increasing evidence on the role of the microbiome in the establishment of auto-immune diseases.

- In germ-free mice models of Th17 cell-dependent arthritis, mono-association with SFB is sufficient to induce disease. A single commensal microbe, via its ability to promote a specific Th cell subset, can drive an autoimmune disease. (68)
- In non-obese, diabetic mice (NOD), models for type 1 diabetes (T1D) in humans, the incidence of T1D is dependent on immune-microbial interaction. Specific pathogen-free NOD mice with a genetic disruption of TLR signaling do not develop T1D. This effect is dependent on commensal microbes because genetically identical but germ-free mice develop robust diabetes, whereas colonization of these germ-free mice with a defined microbial consortium attenuates T1D. (69)

Studies like these provide increasing evidence that the interaction of gut microbiota with the innate immune system might be a vital factor modifying human disease.

Bacteria-bacteria interaction

Beyond host-microbiota interaction, bacteria can also interact with one another. Quorum sensing is a cell-cell communication mechanism through which bacteria count their own numbers by producing and detecting the accumulation of a signaling molecule that they export into their environment. (70) Bacteria use quorum sensing communication circuits to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. (71) Communication also includes the movement of genes between bacterial species and strains. Gene transfer can happen so that virulence genes are transferred from pathogens to commensals, and these may also serve as reservoirs of genes that encode antibiotic resistance. (43)

Stable state

Gut microbiota normally exists in a stable state. This stable state can tolerate a certain amount of perturbation before it changes towards a different equilibrium state. Exogenous factors, such as antibiotics, invasive species or diet, impact the microbiota of the host. For instance, by exposing healthy volunteers to two courses of ciprofloxaxin over a 10-month period, the fecal microbiota reached a stable state similar to but yet distinct from the pre-treatment state. (72) In the same manner, three individuals with dyspepsia given one week of metronidazole, clarithromycin, and omeprazole had a state shift that persisted up to four years without additional antibiotic treatment. (73) In both cases, significant increases in antibiotic-resistance genes persisted for years.

Colonization Resistance

The stability of the gut microbiota is guaranteed by another fundamental role of the microbiota, colonization resistance. It translates the ability of native microbiota to prevent the establishment of harmful or potentially beneficial microbes, by competing for attachment sites and nutrients, and through production and secretion of antimicrobial products. (47,51)

Most studies involving probiotics use a single species or a few species thought to be beneficial. However, probiotic challenges typically do not introduce lasting changes in the host microflora. (74)

In rat models, transplanting exogenous microbiota resulted in a marked increase in the microbial diversity of the recipients. However, when transplantation was performed after antibiotic intake, an increase in the establishment of donor phylotypes was not observed. (75) This study challenges the more intuitive view that the native community

is more resilient to colonization than a less diverse post-antibiotic community. An indigenous gut microbial community might be reshaped to an extent not previously anticipated.

In summary, resilience is a characteristic of healthy microbiota but being resilience doesn't characterize a microbial community as healthy. (47)

Establishment of Adult Microbiota

Although the intrauterine environment is generally considered sterile, recent evidence showed a unique placental microbiota composed of nonpathogenic commensal microbiota. (76) The earliest infant stool sample, the meconium, is free of detectable viral particles and harbors very low diversity of bacteria. Babies are exposed to microbes from different environments immediately upon birth and are rapidly colonized by the microbes they first encounter, either from their mother's vagina in eutocic delivery or from skin microbes in caesarian-section births. Delivery mode has been hypothesized to influence immunological functions during the first year of life via gut microbiota development, with babies delivered by caesarean section having lower bacterial cell counts in fecal samples and a higher number of antibody-secreting cells. (41) Although biological mothers are in a unique position to transmit an initial inoculum of microbes to their infants during and following birth, an analysis of mothers of teenage USA twins showed that their fecal microbiota were no more similar to their children than were those of biological fathers. Furthermore, monozygotic and dizygotic adult twins share equally similar microbiota. Shared environment rather than genes might drive familial similarities, for example, differences in the microbiota and the microbiome may help explain interpersonal variations in gut metabolic processes, including metabolism of drugs and dietary substrate responses. (77)

Besides providing ideal nutrition, breast milk is dense in human milk oligosaccharides and other bioactive molecules that nurture the proliferation of a protective gut bacterial community enriched in bifidobacteria. (78) It has been proposed that suckling might provide vertical mother-infant transfer of microbes not only of skin associated bacteria (through direct contact) but also, through a hypothesized entero-mammary pathway, in which viable maternal gut bacteria reach breast milk. (79)

The diversity of both bacteria and viruses in the infant gut is initially very low, and then climbs through early development. Early colonizers are generally aero-tolerant, as the gut initially contains oxygen, and then are replaced by anaerobes that are typical of the adult gut microbiota. The progressive shifts in community composition seen in

microbiota development are related to changes in its functional profile. The earliest microbiome is enriched in genes facilitating lactate utilization and functional genes involved in plant polysaccharide metabolism are present before the introduction of solid food, preparing the host for dietary change. (41) Phylogenetic diversity increases gradually over time evolving towards an adult-like configuration within the 3-year period following birth. (77) Once the microbiota has reached maturity it remains mostly stable until old age. (41) Adult individuals studied over a long period of time registered a high level of variability in both diversity and community structure, signifying that a mature and stable microbiome does not correspond to a static microbiome. (80) The microbiota composition in the elderly is different from that of young adults. Variability in community composition is greater in this age group than for adults, which could be related to the greater range of morbidities associated with age and the subsequent use of medications to treat them. (41)

Early life is critical for microbiota development

A landmark study on microbial development in mice found that disrupting the microbiota during a critical, early-life, time window resulted in long-term metabolic and immunity changes. Cox and colleagues studied the exposure of mice to low dose penicillin during a specific time window around birth, that is, mother exposure and subsequent weaning period. They found that transient changes to the microbiota had a sex-specific, long-term effect on body composition. These changes included increased total mass, fat mass, increased hepatic expression of genes involved in adipogenesis as well as others. The metabolic changes evidenced were not caused by direct effects of antibiotics but rather by derived changes in the gut microbiota. (81) In a different study, germ-free mice accumulate invariant natural killer T cells in the colonic lamina propria and lung, increasing morbidity in models of IBD and allergic asthma. Colonization of neonatal-but not adult-GF mice with a conventional microbiota protected the animals, indicating that early childhood is a critical time window for contact with microbiota. (82)

Both of these studies suggest a strong role of the microbiota in establishing long-term effects on immunity and metabolism during infancy. In a recent study reflecting this concern, investigators were able to partially restore microbiota of cesarean-born infants via vaginal microbial transfer immediately after birth. Although a follow-up longitudinal study is necessary, the objective of the study to establish proof of principle for this technique was a success. (83)

2) Eubiosis and Dysbiosis

A healthy microbiota is defined by high diversity and an ability to resist change under physiological stress. In contrast, microbiota associated with disease, dysbiosis, is defined by lower species diversity, fewer beneficial microbes and/or the presence of commensals that have the potential to cause harm (pathobionts). (84)

Shifts in microbial community composition can destroy the mutualistic relationship between host and microbiota, compromising human health status. Intestinal dysbiosis has been linked with important human diseases, including autoimmune and/or inflammatory disorders, such as IBD, metabolic disorders, such as, obesity, type 2 diabetes, as well as allergies, and neurological disorders. (51)

In genetically susceptible hosts, the changes in the gut microbiota composition could have a profound impact on chronic disease development. For example, a study by Eun *et al* was able to trigger colonic inflammation in all germ-free, IL10 deficient mice belonging to two distinct strains after colonization with an IBD-associated bacterial consortium. (85)

In the case of gut ecology, the immense number of species and strains and the complex relationships between them, the environment and the host make it difficult to ascertain if a specific pathogen is associated with a specific disease, that is, Koch's postulate. (84) Although no single species acts as a biomarker of dysbiosis, some consistent alterations in specific disease states have been described such as *Faecalibacterium prausnitzii* in IBD (86) or *Akkermansia muciniphila* in DM2 (87)**.**

The associations described for these species are not in any chance ubiquitous. In the case of both these species, a recent review highlighted several studies depicting contradictory or even mutually exclusive associations. The authors suggested that strains of *F. prausnitzii,* that have distinct metabolic impacts in the gut system, were inseparable by current rRNA-based classification. (62)

The Human Microbiome Project has shown that despite significant taxonomic variation, there is a similarity in the functional profile of the microbiome of healthy individuals. (44) As such, instead of key bacterial taxa, identifying specific microbial gene or metabolic markers associated with health and disease might serve as potential diagnostic markers. For instance, reduction of *Rumicoccaceae* and *Lachnospiraceae* family microbiota, SCFA producers, in CDI can be interpreted as depletion in butyrate-producing bacteria. (33,88) Also, *Roseburia intestinalis* has been shown to have negative correlations with subcutaneous adiposity, body weight, liver weight, serum

insulin, FGF21 levels, and inflammation markers in mice as well as similar alterations in humans, probably due to its role as a butyrate producer. (89)

Diversity as a Marker of Eubiosis

Microbial diversity seems to be a good a marker of eubiosis as gut communities of lower diversity are often associated with disease phenotypes in humans. (90)

Body sites with restricted microbial diversity in the healthy state such as the esophagus and the vagina might not tolerate increased microbial diversity without adverse consequence, for instance in triggering the development of bacterial vaginosis and chronic esophageal inflammation and cancer. (43) Several publications describe the differences between the microbial composition of humans from developed countries and cohorts representing nonmodern societies. A common finding in all these studies is the lowered bacterial diversity in the developed country group. (91)

For example, differences were found between the composition and gene function between the least diverse USA microbiome and the more diverse Malawian/Amerindian microbiomes.(77) The authors proposed that the western lifestyle (diet) affected the bacterial component of the gut microbiota by obviating the need for a wide diversity of microorganisms needed to utilize the many fibers and other nutrients of a more plant-nutrient rich diet.

The mechanisms that link microbiota diversity and health are still unknown, but SCFAs appear to be one likely mediator. A modern diet is poor in microbiota-accessible carbohydrates (MACs) and as such it is poor on beneficial microbial metabolites. The term MAC was proposed by Sonnenburg and Sonnenburg (91) allows a better definition for carbohydrates destined to microbial fermentation. Terms such as fiber that encompass bulking, non-fermenting agents or plant polysaccharides that dismiss nonplant carbohydrates should be used sparingly. A diet low in exogenous MACs induces reduced microbial diversity by selecting a group of species that are more apt in using host-derived MACs, such as mucin glycans.

3) Diet shapes the microbiota

Diet shapes the microbiota in mice

In order to establish the relative importance of diet in shaping the gut microbiota, investigators fed five inbred strains of mice deficient for genes relevant for host-microbiota interaction as well as a group of outbred mice a HF/HS diet. In all groups there was a consistent change in microbial community that was characteristically rapid, reproducible, and reversible. This study illustrates the importance of diet when compared to genetic associations in shaping the gut microbiota. (92)

Ridaura *et al* studied germ-free mice colonized with flora from obesity-discordant human twins. Their study found that adiposity is transmissible from human to mouse subjects, that is, transplantation of the human obese twin transmitted the obese phenotype to the mice. Also, they showed that, by cohousing the two groups of (coprophagic) mice, that is, the mice transplanted with the lean twin's microbiota (Ln) and the mice transplanted with the obese twin's microbiota (Ob), that the latter resisted the development of obesity. This phenotype rescue correlated with the invasion of Bacteroidales species originating in the Ln mice and was only evident in mice fed a diet lower in saturated fats and high in food and vegetables. These results reveal that transmissible and modifiable interactions between diet and microbiota influence host biology. (93)

Another murine study highlights the reasoning that in some genetic backgrounds, environmental reshaping of the microbiota can significantly improve the development of metabolic syndrome features while in others, host-related factors might be dominant. The same mouse strain, originating from different vendors was compared. The 129T strain from Taconic Farms is usually susceptible to developing diet-induced obesity and the 129J from Jackson laboratories is resistant to the development of metabolic syndrome. When these two groups of genetically identical mice are co-housed and fed the same diet (environmental normalization), the 129T becomes metabolic syndrome resistant after 3 generations. On the other hand, a different strain of metabolic syndrome prone B6J mice housed under the same conditions acquired similar microbiota but did not change its phenotype. (89)

Diet shapes the microbiota in humans

A controlled-feeding study of 10 subjects showed that microbiome composition changed detectably within 24 hours of initiating a high-fat/low-fiber or low-fat/high-fiber diet, but that its overall microbial identity (enterotype) remained stable during the

10-day study. Thus, alternative enterotypes might be associated with long-term diet. (94) Long-term dietary interventions may allow modulation of an individual's enterotype to improve health.

Consumption of diets composed entirely of animal or plant products alters microbial community structure in just a few days. The animal-based diet increased the abundance of bile-tolerant microorganisms and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides, reflecting trade-offs between carbohydrate and protein fermentation. Finally, increases in the abundance and activity of *Bilophila wadsworthia* on the animal-based diet support a link between dietary fat, bile acids, and the outgrowth of microorganisms capable of triggering inflammatory bowel disease. (95)

Dietary intervention in a group of 49 overweight or obese individuals resulted in loss of fat mass and was accompanied by improvements in clinical markers such as lipid and insulin values, insulin resistance, and measures of inflammation for both the LGC and HGC groups. The LGC cohort also registered an increase in gene richness that approached but still remained significantly lower than the HGC. However this improvement was less efficient for inflammation variables in individuals with lower gene richness. Low gene richness may therefore have predictive potential for the efficacy of dietary intervention. (96)

Another study comparing elite athletes to normal weight and overweight/obese individuals found that, microbiota diversity indices positively correlated with protein intake and creatine kinase suggesting that diet and exercise are drivers of biodiversity in the gut. The protein and microbiota diversity relationship was further supported by a positive correlation between urea levels, a by-product of diets that are rich in protein, and microbiota diversity. (97)

In humans the available evidence would suggest that the differences in the gut microbiota are associated with obesity probably result from the utilization of a high-calorie, high-fat and high-carbohydrate Western diet. (98) The composition of the human gut microbiota is rather stable and short-term dietary interventions do not profoundly change the microbiota composition. Nonetheless, the microbial gene expression and therefore the functional profiles seem to adapt to changes in diet rapidly. (99)

The knowledge of the mechanisms by which changes in the microbiome occur allows us to foresee changes induced by exogenous sources. Changes in diet are the seemingly

most accessible manner of manipulating the microbiota in order to improve human health and to treat or prevent disease.

Diet, microbiota and drugs

Diet induced changes may also play a role in pharmacokinetic pathways. *Eggerthella lenta* is a gut colonizing Actinobacterium that can transform digoxin into the inactive metabolite, dihydrodigoxin. By expanding on the knowledge that arginine inhibits this reaction *in vitro*, investigators found that by increasing the dietary protein in germ-free mice colonized with *E. lenta* the serum and urine digoxin concentration significantly increased. Identification of gut microbial biomarkers through rapid qPCR-based as well as dietary adjustments could be able to guide dosage schemes. (100)

Possible limitations to dietary efforts: Long-term effects of dietary changes in human populations

The changes of the microbial membership of calcified microbial dental plaque in European skeletons allowed the identification of periods in history corresponding to the largest shifts in the oral microbial community. The transition from hunter-gatherer to farming shifted the oral microbial community to a disease-associated configuration. The composition of oral microbiota remained unexpectedly constant between neolithic and medieval times, after which (the now ubiquitous) cariogenic bacteria became dominant, apparently during the Industrial Revolution. Modern oral microbial ecosystems are markedly less diverse than historic populations, which might be contributing to chronic oral (and other) disease in postindustrial lifestyles. These two periods correspond to the greatest dietary shifts in human evolution. (101)

To test the hypothesis that a diet low on microbiota-accessible carbohydrates causes a decrease in microbiota diversity, mice colonized with human microbiota were switched from a high-MAC diet to a low-MAC diet for 7 weeks. A significant reduction in diversity was noted. By switching back to a high-MAC diet, there was a partial recovery of diversity markers. The low-MAC diet perturbation induced permanent loss of diversity on the microbiota. The investigators then bred both the control group and the test group to understand if this effect was magnified over generations. Every generation, the descendants were weaned onto the respective diet of their parents. After reproducing the new parents were then switched to a high-MAC diet. With each new generation the low-MAC diet resulted in a progressive loss of diversity that was progressively less recoverable with introduction of a high-MAC diet. This model suggests that over several generations there were increasing, irreversible extinctions of microbial species

due to a low abundance of MAC in the diet. By the fourth generation, mice in the test group were transplanted (through gavage) with fecal samples from fourth generation controls. This resulted in the reestablishment of bacterial diversity. (102)

4) The impact of Gut Microbiota on a common "western" disease: Obesity

The western diet and lifestyle is associated with loss of gut microbial diversity and the appearance of western diseases the most frequent of which is obesity. Over the following pages the main evidence proving this association will be exposed.

Gut microbiota is altered in obesity

Several changes have been reported in regards to the microbiome in overweight and obese individuals. These changes are probably not a mere consequence of obesity. In mice, the obese phenotype can be transmitted by gut microbiota transplantation, indicating that gut microbial populations may have an active role in the development of obesity. (103) A number of studies have demonstrated gut composition differences between obese and lean human subjects. The most reported alteration is a reduction in the Bacteroidetes:Firmicutes proportion. A meta-analysis reviewing these results found that the only reproducible and significant alteration at the phylum level is a decrease in the absolute number of sequences of Firmicutes in the obese subject's group. At a genus levels there was significantly depletion in Bifidobacteria, which are members of the Actinobacteria phylum. (104)

Microbial diversity in obesity

The importance of microbial diversity has been identified as an important factor related to obesity. When tested for bacterial richness, a group of 292 individuals split into two groups; one consisting of a low-gene-count and a high-gene-count group representing lower and higher microbial diversity respectively. The LGC group included a significantly higher proportion of obese participants and was characterized by more marked adiposity associated with increased serum leptin, decreased serum adiponectin, insulin resistance, hyperinsulinemia, increased levels of triglycerides and free fatty acids, decreased HDL-cholesterol and a more marked inflammatory phenotype.

The investigators found that the changes in the metabolic profile of the LGC group suggested that this group harbors an inflammation-associated microbiota. These changes included: a) a reduction of butyrate-producing bacteria; b) increased mucus degradation potential c) reduced hydrogen and methane production potential combined with increased hydrogen sulphide formation potential; d) an increase in *Campylobacter*/*Shigella* abundance; and e) an increased potential to manage oxidative stress. (90)

Mechanisms linking microbiota and obesity

Early research suggested that the increase in body weight associated with gut microbiota was due to an increased capacity of the microbiota to extract nutrients from the diet as well as induced changes in the metabolism of the host (e.g. increased fatty acid oxidation and triglyceride storage in the liver). (105,106)

These studies were performed by comparing the difference in weight gain between germ-free mice and conventionally raised mice groups. However, another study (107) found no significant difference in weight gain between GF and CONV groups under low fat diet, reporting no linkage between gut microbiota and diet-induced obesity.

Although these statements might represent a conflicting image, when critically interpreted we find important confounding factors. Dietary formulations presenting a similar macronutrient balance but different sources of carbohydrates and fat, as well as differences in mice strains are prevalent across GF and CONV mice comparison studies. (62)

Overall, GF-CONV comparisons do find differences in energy metabolism between the two groups. These differences, although not ubiquitous, link microbial presence to obesity. (62)

Suppression of *Fiaf* as a probable mechanism

The first studies to explore the causal mechanism between this association proposed that the microbial colonization of the gut introduced more calories through processing of dietary polysaccharides and that suppressed *Fiaf (*Fasting induced adipocyte factor) activity in the gut epithelium led to increased LPL activity in adipocytes, promoting TG storage of calories harvested from the diet. (106,108) More recently, the paradigm shifted and inflammation is thought to be the most relevant mechanism regarding this association.

The role of gut inflammation and diet

Obesity and associated metabolic disorders are characterized by chronic or low-grade inflammation. (109) Bacterial lipopolysaccharide (LPS), a breakdown product of the outer membrane of Gram-negative staining bacteria, has been proposed as a triggering factor for the increased inflammatory process in obese individuals and diet seems to play an important role in starting that process. Mice fed with a high-fat diet demonstrate augmented plasma LPS at a concentration sufficient to increase body weight, fasted glycemia, and inflammation. Accordingly, LPS infusion in normal diet-fed mice raised a metabolic response similarly to high-fat feeding. The described metabolic

endotoxemia was characterized as a 2 to 3-fold increase in serum LPS. This increase was still 10–50 times lower than values that could be reached during sepsis or other infections. (110) Changes in inflammation include an increase in intestinal permeability, plasma LPS, as well as increased phosphorylation of myosin light chain, alteration in tight-junction proteins and an altered cellular distribution of occludin. (111)

A high-fat diet triggers changes in the gut microbiota, but it is the development of inflammation that is associated with the obese phenotype

By comparing obesity-prone and obesity-resistant mice strains fed the same high-fat diet researchers found that diet caused similar changes in gut microbiota composition in both groups but only in the obese-prone group were inflammation markers increased.(111)

In another study, female RELM null mice, harboring a genetic resistance to intestinal inflammation, were found to be resistant to the obesogenic effects of a high-fat diet. Even so, they were susceptible to changes in the gut microbiota similar to wild-type mice, proving that diet modifies gut microbiota independently from obesity.(112)

Yet another study evaluated the impact of a high-fat/high-sugar diet in a murine model. The investigators concluded that this diet created a specific inflammatory environment in the gut, correlated with intestinal mucosa dysbiosis characterized by an overgrowth of pro-inflammatory Proteobacteria such as *E. coli*, a decrease in protective bacteria, and a significantly decreased of SCFA concentrations. They added that mice treated with an agonist of GPR43 (a SCFA receptor reduced in dysbiosis) were protected against chemical-induced colitis. The depletion of fiber generally associated with the western type diet could partially explain the decrease of SCFA concentrations, but cannot explain the impact of HF/HS diet on global gut dysfunction by itself. (113)

A more complex approach on the mechanism

A more integrated view on the mechanism behind the diet-microbiome-host relationship proposes a more complex approach. A recent review postulated that microbes whose competitive advantage is dependent on anaerobic respiration, such as Proteobacteria, adopt a pro-inflammatory life history strategy which results in increased nitrate and that their competitors promote mucosal homeostasis (which limits nitrate). (62)

Common pathology-associated microbial species seem to thrive in inflammation. A recent study showed that nitrate generated as a by-product of the host inflammatory response can be utilized by *E.coli*. Obligate anaerobic microbes such as those belonging to the Firmicutes and Bacteroidetes phyla, compete for nutrients that are available for

fermentation, originating SCFAs, but cannot utilize non-fermentable nutrients. Reduced levels of butyrate, starving the epithelial cells as well as decreasing the number of TReg cells, promote inflammation. Unlike obligate anaerobic members of the gut microbiota, the facultative anaerobic *Enterobacteriaceae* can use nitrate, S-oxides and N-oxides as terminal electron acceptors for anaerobic respiration. The ability to degrade non-fermentable substrates likely enables *E. coli* to bypass its competition, which explains the fitness advantage conferred by nitrate respiration in the inflamed gut. (114)

Another important host factor in this relationship is bile secretion. Bile has potent antimicrobial properties that can contribute to the selection or exclusion of many potential gut microbiota.(115)

For instance, *Bilophila wadsworthia*, a sulphite-reducing human pathobiont, is known to flourish in the presence of taurine-conjugated bile acid (a rich source in organic sulphur). Researchers found that saturated fats induced a bloom in *B. wadsworthia* that was dependant on a host-derived response by means of increased taurocholic acid production. Several intestinal pathogens are not only bile-resistant, but highly favored in the presence of bile. (116)

Cholic acid regulates the composition of gut microbiota in rats, inducing similar changes to those induced by high-fat diets. Higher amounts of bile acids are also linked to lower fecal concentrations of butyrate. This finding suggests bile acids either select against the proliferation of butyrate producing bacteria or inhibit the metabolic pathways leading to butyrate synthesis. (117) Western-style diets have reduced intake in fermentable polysaccharides and are associated with lower levels of SCFAs. Fat types that specifically promote taurocholate may exacerbate the inflammatory processes since they are strongly linked to expansion of sulfate-reducing bacteria and production of H_2S enhances the competitiveness of bacteria that drive systemic inflammation via LPS.

Dietary intervention acts on the obesity-related microbiota

Weight loss on two types of low-calorie diet increased the lowered Bacteroidetes:Firmicutes proportion found in most obese individuals. (118) In a recent study, diet with added fiber also shifted the Bacteroidetes:Firmicutes ratio toward that previously associated with healthy lean subjects, and this was independent of caloric restriction. Added fiber altered microbial community structure rather than introducing or removing existing community members. Shifts in bacterial gene abundances after fiber supplementation included genes associated with carbohydrate, amino acid, and lipid metabolism, as well as metabolism of cofactors and vitamins. Nonetheless, several

taxonomic shifts would argue against benefits of fiber, such as reduction of known butyrate producers and fecal butyrate concentrations. (119)

Using microbiota targeting therapies in obesity

Rebalancing the gut microbiota seems to be a possible manner to intervene in metabolic syndrome patients. Most trials that focus on using probiotics to modify human metabolism use *Lactobacillus* and/or *Bifidobacterium* strains. These are fermentative species whose community-level impact on the flora might be limited. Other limitations of such trials are small sample sizes and absence of longer-term follow up. Results are underwhelming. (120) In theory, as exposed earlier in this review, the ideal age to enhance ideal microbiota establishment would be during its development. Even so, only two randomized controlled trials regarding early probiotic use for metabolic syndrome have been published with no significant long-term results having being detected. (121)

In 2012 the only study regarding fecal microbiota transplantation and metabolic syndrome was published. Eighteen male individuals were screened for metabolic syndrome (30 kg/m^2 or waist circumference > 102 cm and a fasting plasma glucose level >5.6 mmol/L), randomized, and allotted in two groups: one receiving an autologous transplant and the other a transplant from a lean donor. The results of this double-blind controlled trial showed an improvement in insulin sensitivity along with an increase in butyrate producing bacteria and increased gut microbiota diversity.(122)

It seems unlikely that fecal transplantation performed through invasive methods could be used routinely for the treatment of obesity, metabolic syndrome or other non-acute diseases. As advances in the procedure are pushed forward in clinical research aimed at diseases such rCDI, the use of more controlled inoculums as in bacteriotherapy, that is, inoculums consisting of selected specific bacterial groups would arise.

Conclusion

The gut microbiota's role in human homeostasis is more complex than expected. As the knowledge what concerns its functions becomes apparent, the increasing diversity of applicabilities becomes tempting. Limitations in current technology such as the inability to differentiate between potentially different bacteria belonging to the same phylotype, or even less than ideal sampling efforts, might allow a certain distortion of results that further research could elucidate. Instead of using bacteria associated with fermentation, FMT modifies the recipient's microbiota using a community of organisms isolated from a healthy gut. The knowledge gained from the use of FMT for the treatment of recurrent *C. difficile* infection may pave the way for modern therapies acting on the microbial component of many diseases.

Shaping the adult microbiome has its difficulties due to its inherent stability and resistance to change. Acting in a defining timeframe such as the perinatal period might be the key for optimal microbiota development.

It is now evident that diet plays a fundamental role in shaping the gut microbiome. Presumed dietary-driven historical microbial extinctions may be responsible for the disappearance of symbiotic microbes in modern populations. Also, the high-fat, high-sugar and low-fiber diet clearly partakes in the lower diversity now commonly observed in the industrialized world. The disruption of host-microbiota interaction, termed dysbiosis, is linked to several "western" diseases including obesity. Even though a causal relationship between microbiota and obesity in humans hasn't been clearly established, evidence for its linkage is increasing. Restoring inaccessible, potentially beneficial microbes alongside dietary changes might be the future of personalized medicine.

Annex

Protocol for Fecal Microbiota Transplantation

Donors (<60 years of age) were volunteers who were initially screened using a questionnaire addressing risk factors for potentially transmissible diseases. Donor feces were screened for parasites (including *Blastocystis hominis* and *Dientamoeba fragilis*), *C. difficile*, and enteropathogenic bacteria. Blood was screened for antibodies to HIV; human T-cell lymphotropic virus types 1 and 2; hepatitis A, B, and C; cytomegalovirus; Epstein–Barr virus; *Treponema pallidum*; *Strongyloides stercoralis*; and *Entamoeba histolytica*. A donor pool was created, and screening was repeated every 4 months. Before donation, another questionnaire was used to screen for recent illnesses. Feces were collected by the donor on the day of infusion and immediately transported to the hospital. Feces were diluted with 500 ml of sterile saline (0.9%). This solution was stirred, and the supernatant strained and poured in a sterile bottle. Within 6 hours after collection of feces by the donor, the solution was infused through a nasoduodenal tube (2 to 3 minutes per 50 ml). The tube was removed 30 minutes after the infusion, and patients were monitored for 2 hours. For patients who had been admitted at referring hospitals, the donor-feces solution was produced at the study center and immediately transported and infused by a study physician. (8)

References

1. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, et al. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: "RePOOPulating" the gut. Microbiome. 2013;1(1):3.
2. Tedesco FJ, Stanley RJ, Alpers DH. Diagnostic Features of Clindamycin- Associated Pseudomembranous Colitis. N Engl J Med. 1974;290(15):841–3.
3. Slimings C, Riley T V. Antibiotics and hospital-acquired Clostridium difficile infection: Update of systematic review and meta-analysis. Journal of Antimicrobial Chemotherapy. 2014;69(4):881–91.
4. Bingley PJ, Harding GM. Clostridium difficile colitis following treatment with metronidazole and vancomycin. Postgrad Med J. 1987;63(745):993–4.
5. Leffler DA, Lamont JT. Clostridium difficile Infection. N Engl J Med. 2015;372(16):1539–48.
6. Nylund CM, Eide M, Gorman GH. Association of clostridium difficile infections with acid suppression medications in children. J Pediatr. 2014;165(5):979–84
7. Debast SB, Bauer MP, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect. European Society of Clinical Infectious Diseases; 2014;20 Suppl 2:1-26
8. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368(5):407–15.
9. Norén T, Åtkerlund T, Bäck E, Sjöberg L, Persson I, Alriksson I, et al. Molecular epidemiology of hospital-associated and community-acquired Clostridium difficile infection in a Swedish county. J Clin Microbiol. 2004;42(8):3635–43.
10. Barbut F, Richard A, Hamadi K, Chomette V, Burghoffer B, Petit JC. Epidemiology of recurrences or reinfections of Clostridium difficile- associated diarrhea. J Clin Microbiol. 2000;38(6):2386–8.
11. Bowman KA, Broussard EK, Surawicz CM, Surawicz C. Fecal microbiota transplantation: current clinical efficacy and future prospects. Clin Exp Gastroenterol. 2015;8:285–91.
12. Eiseman B, Silen W, Bascom G, Kauvar A. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44(5):854–9.
13. Hafiz S, Oakley CL. Clostridium difficile isolation and characteristics. J Med Microbiol. 1976;9(2):129–36.
14. Schwan A, Sjölin S, Trottestam U, Aronsson B. Relapsing Clostridium difficile enterocolitis cured by rectal infusion of normal faeces. Scand J Infect Dis. 1984;16(2):211–5.
15. Brandt LJ, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, Park T, et al. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. Am J Gastroenterol. Nature Publishing Group; 2012;107(7):1079–87.
16. Li Y-T, Cai H-F, Wang Z-H, Xu J, Fang J-Y. Systematic review with meta-analysis: long-term outcomes of faecal microbiota transplantation for Clostridium difficile infection. Aliment Pharmacol Ther. 2015;1–13.
17. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature. Nature Publishing Group; 2015;517(7533):205–8.
18. Theriot CM, Koenigsknecht MJ, Carlson PE, Hatton GE, Nelson AM, Li B, et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. Nat Commun. 2014;5:3114.
19. Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol. 2014;306(4):G310–9.
20. Francis MB, Allen CA, Shrestha R, Sorg JA. Bile Acid Recognition by the Clostridium difficile Germinant Receptor, CspC, Is Important for Establishing Infection. PLoS Pathog. 2013;9(5).
21. Schenck LP, Beck PL, MacDonald JA. Gastrointestinal dysbiosis and the use of fecal microbial transplantation in Clostridium difficile infection. World J Gastrointest Pathophysiol. 2015;6(4):169–80.
22. Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for Clostridium difficile spores. J Bacteriol. 2008;190(7):2505–12.
23. Sorg JA, Sonenshein AL. Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid. J Bacteriol. 2010;192(19):4983–90.
24. Giel JL, Sorg JA, Sonenshein AL, Zhu J. Metabolism of bile salts in mice influences spore germination in clostridium difficile. PLoS One. 2010;5(1).
25. Cai JS, Chen JH. The Mechanism of Enterohepatic Circulation in the Formation of Gallstone Disease. J Membr Biol. 2014;247(11):1067–82.
26. Rupnik M. Toward a True Bacteriotherapy for Clostridium difficile Infection. N Engl J Med. 2015;37216(16):1566–8.
27. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutation Research - Reviews in Mutation Research. 2005;589(1):47–65.
28. Alang N, Kelly CR. Weight Gain After Fecal Microbiota Transplantation. Open Forum Infect Dis. 2015;2(1):1–8.
29. Solari P, Fairchild P, Noa L, Wallace M. Tempered Enthusiasm for Fecal Transplant. Clin Infect Dis. 2014;59(2):319.
30. Baxter M, Ahmad T, Colville A, Sheridan R. Fatal aspiration pneumonia as a complication of fecal microbiota transplant. Clin Infect Dis. 2015;61(1):136–7.
31. Weingarden A, González A, Vázquez-Baeza Y, Weiss S, Humphry G, Berg-Lyons D, et al. Dynamic changes in short- and long-term bacterial composition following fecal microbiota transplantation for recurrent Clostridium difficile infection. Microbiome. 2015;3(1):10.
32. Fuentes S, van Nood E, Tims S, Heikamp-de Jong I, Ter Braak CJ, Keller JJ, et al. Reset of a critically disturbed microbial ecosystem: faecal transplant in recurrent Clostridium difficile infection. ISME J. Nature Publishing Group; 2014;8(8):1621–33.
33. Song Y, Garg S, Girotra M, Maddox C, Von Rosenvinge EC, Dutta A, et al. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. PLoS One. 2013;8(11):1–11.
34. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. Jama. 2014;312(17):1772–8.
35. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. Jama. 2016;315(2):142–9.
36. Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, et al. Fecal microbiota transplant for relapsing clostridium difficile infection using a frozen inoculum from unrelated donors: A randomized, open-label, controlled pilot study. Clin Infect Dis. 2014;58(11):1515–22.
37. Hirsch BE, Saraiya N, Poeth K, Schwartz RM, Epstein ME, Honig G. Effectiveness of fecal-derived microbiota transfer using orally administered capsules for recurrent Clostridium difficile infection. BMC Infect Dis. BMC Infectious Diseases; 2015;15:191.

38. Varier RU, Biltaji E, Smith KJ, Roberts MS, Kyle Jensen M, LaFleur J, et al. Cost-Effectiveness Analysis of Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection. Infect Control Hosp Epidemiol. 2015;36(4):438-44.
39. Zainah H, Hassan M, Shiekh-Sroujieh L, Hassan S, Alangaden G, Ramesh M. Intestinal microbiota transplantation, a simple and effective treatment for severe and refractory Clostridium difficile infection. Dig Dis Sci. 2015;60(1):181–5.
40. Tvede M, Rask-Madsen J. Bacteriotherapy For Chronic Relapsing Clostridium Difficile Diarrhoea In Six Patients. Lancet. 1989;333(8648):1156–60.
41. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: An integrative view. Cell. Elsevier Inc.; 2012;148(6):1258–70.
42. Belizário JE, Napolitano M. Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. Front Microbiol. 2015;6:1–16.
43. Pflughoeft KJ, Versalovic J. Human microbiome in health and disease. Annu Rev Pathol Mech Dis. 2012;7:99–122.
44. Human Microbiome Project Consortium T. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14.
45. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174–80.
46. Fischbach MA, Sonnenburg JL. Eating for two: How metabolism establishes interspecies interactions in the gut. Cell Host and Microbe. 2011;10(4):336–47.
47. Lozupone C, Stomabaugh J, Gordon J, Jansson J, Knight R. Diversity , stability and resilience of the human gut microbiota. Nature. 2012;489(7415):220–30.
48. Louis P, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environ Microbiol. 2010;12(2):304–14.
49. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. Science (80-). 2012;336(6086):1262–7.
50. Theriot CM, Young VB. Microbial and metabolic interactions between the gastrointestinal tract and Clostridium difficile infection. Gut Microbes. 2014;5(1):86–95.
51. Schippa S, Conte MP. Dysbiotic events in gut microbiota: Impact on human health. Nutrients. 2014;6(12):5786–805.
52. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489(7415):242–9.
53. McNeil NI. The contribution of the large intestine to energy supplies in man. Am J Clin Nutr. 1984;39(2):338–42.
54. Okeke F, Roland BC, Mullin GE. The Role of the Gut Microbiome in the Pathogenesis and Treatment of Obesity. Glob Adv Heal Med. 2014;3(3):44–57.
55. Evans JM, Morris LS, Marchesi JR. The gut microbiome: The role of a virtual organ in the endocrinology of the host. Journal of Endocrinology. 2013;218(3):37-47
56. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, Tryptophan Metabolism and the Brain-Gut- Microbiome Axis. Behav Brain Res. 2014;277:1–17.
57. Zhou L, Foster JA. Psychobiotics and the gut – brain axis : in the pursuit of happiness. Neuropsychiatr Dis Treat. 2015;11:715–23.
58. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: A gut microbiota perspective. Current Opinion in Biotechnology. 2013;24(2):160–8.
59. Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. International Journal of Pharmaceutics. 2008;363(1-2):1–25.
60. Watabe T, Okuda H, Ogura K. Lethal drug interactions of the new antiviral, sorivudine, with anticancer prodrugs of 5-fluorouracil. Yakugaku Zasshi. 1997;117(10-11):910–21.
61. Hooper L V, Littman DR, Macpherson AJ, Program MP. Interactions between the microbiota and the immune system. Science. 2012;336(6086):1268–73.
62. Ha CW, Lam YY, Holmes AJ. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. World J Gastroenterol. 2014;20(44):16498–517.
63. Kim B-S, Jeon Y-S, Chun J. Review: Current status and future promise of the human microbiome. Pediatr Gastroenterol Hepatol Nutr. 2013;16(2):71–9.
64. Mcdermott AJ, Huffnagle GB. The microbiome and regulation of mucosal immunity. Immunology. 2014;142(1):24–31.
65. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011;331(6015):337–41.
66. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011;332(6032):974–7.
67. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. Cell. 2009;139(3):485–98.
68. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010;32(6):815–27.
69. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008;455(7216):1109–13.
70. Bassler BL, Losick R. Bacterially Speaking. Cell. 2006;125(2):237–46.
71. Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol. 2001;55:165–99.
72. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A. 2011;108 Suppl :4554–61.
73. Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One. 2010;5(3):e9836.
74. Lawley TD, Walker AW. Intestinal colonization resistance. Immunology. 2013;138(1):1–11.
75. Manichanh C, Reeder J, Gibert P, Varela E, Llopis M, Antolin M, et al. Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. Genome Res. 2010;20(10):1411–9.
76. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014;6(237):237-65.
77. Gordon J, Yatsunenko T, Rey F, Manary M, Trehan I, Dominguez-Bello M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222–7.
78. Martin MJ, Vazquez E. Building a Beneficial Microbiome from Birth. Adv Nutr. 2016;7:323–30.
79. Jost T, Lacroix C, Braegger CP, Rochat F, Chassard C. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environ Microbiol. 2014;16(9):2891–904.
80. Flores GE, Caporaso JG, Henley JB, Rideout JR, Domogala D, Chase J, et al. Temporal variability is a personalized feature of the human microbiome. Genome Biol. 2014;15(12):531.
81. Cox LM, Yamanishi S, Sohn J, Alekseyenko A V., Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158(4):705–21.
82. Olszak T, An D, Zeissig S, Vera MMP, Richter J, Franke A, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science. 2012;336(6080):489–93.

83. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, et al. Partial Restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med. 2016;22(3):250-3
84. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol. 2014;16(7):1024–33.
85. Eun CS, Mishima Y, Wohlgemuth S, Liu B, Bower M, Carroll IM, et al. Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-$10^{-/-}$ mice. Infect Immun. 2014;82(6):2239–46.
86. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of faecalibacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis. 2009;15(8):1183–9.
87. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490(7418):55–60.
88. Antharam VC, Li EC, Ishmael A, Sharma A, Mai V, Rand KH, et al. Intestinal dysbiosis and depletion of butyrogenic bacteria in Clostridium difficile infection and nosocomial diarrhea. J Clin Microbiol. 2013;51(9):2884–92.
89. Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, et al. Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome. Cell Metab. 2015;22(3):516–30.
90. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500(7464):541–6.
91. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: The deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. Cell Metab. 2014;20(5):779–86.
92. Carmody RN, Gerber GK, Luevano JM, Gatti DM, Somes L, Svenson KL, et al. Diet dominates host genotype in shaping the murine gut microbiota. Cell Host Microbe. 2015;17(1):72–84.
93. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau L, et al. Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice Gut Microbiota from Twins Metabolism in Mice. Science. 2013;341(6150):1241214.
94. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334(6052):105–8.
95. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559–63.
96. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, et al. Dietary intervention impact on gut microbial gene richness. Nature. 2013;500(7464):585–8.
97. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, et al. Exercise and associated dietary extremes impact on gut microbial diversity. Gut. 2014;63(12):1913-20
98. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, et al. Gut microbiota, obesity and diabetes. Postgrad Med J. 2016;92(1087):286-300
99. Graf D, Di Cagno R, Fåk F, Flint HJ, Nyman M, Saarela M, et al. Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis. 2015;26:26164.
100. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and Manipulating Cardiac Drug Inactivation by the Human Gut Bacterium Eggerthella lenta. Science. 2013;341(6143):295–8.
101. Adler CJ, Dobney K, Weyrich LS, Kaidonis J, Walker AW, Haak W, et al. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat Genet. 2013;45(4):450–5, 455e1.
102. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. Nature. 2016;529(7585):212–5.
103. Turnbaugh PJ, Ley RE, Mahowald M a, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.
104. Angelakis E, Armougom F, Million M, Raoult D. The relationship between gut microbiota and weight gain in humans. Future Microbiol. 2012;7(1):91–109.
105. Ley R, Turnbaugh P, Klein S, Gordon J. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022–3.
106. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. World J Gastroenterol. 2014;20(43):16079–94.
107. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr. 2010;104(06):919–29.
108. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci. 2007;104(3):979–84.
109. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860–7.
110. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761–72.
111. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE, et al. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol - Gastrointest liver Physiol. 2010;299(2):G440–8.
112. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, et al. High-Fat Diet Determines the Composition of the Murine Gut Microbiome Independently of Obesity. Gastroenterology. 2009;137(5):1716-24
113. Agus A, Denizot J, Thévenot J, Martinez-Medina M, Massier S, Sauvanet P, et al. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation. Sci Rep. 2016;6:19032.
114. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, et al. Host-derived nitrate boosts growth of E. coli in the inflamed gut. Science. 2013;339(6120):708–11.
115. Hofmann AF, Eckmann L. How bile acids confer gut mucosal protection against bacteria. Pnas. 2006;103(10):4333–4.
116. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature. 2012;487(7405):104–8.
117. Islam KBMS, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology. 2011;141(5):1773–81.
118. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022-3
119. Holscher HD, Caporaso JG, Hooda S, Brulc JM, Fahey Jr. GC, Swanson KS. Fiber supplementation influences phylogenetic structure and functional capacity of the human intestinal microbiome: follow-up of a randomized controlled trial. Am J Clin Nutr. 2015;101(1):55–64.
120. Kobyliak N, Conte C, Cammarota G, Haley AP, Styriak I, Gaspar L, et al. Probiotics in prevention and treatment of obesity: a critical view. Nutr Metab. 2016;13(1):14.
121. Balasubramanian H. Early probiotics to prevent childhood metabolic syndrome: A systematic review. World J Methodol. 2015;5(3):157.
122. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012;143(4):913–6.e7.